HPV infection
HPV infection cycle is linked to keratinocyte differentiation program

Normal viral life cycle

Viral protein expression

HPV virion
Episomal DNA
Early proteins
Late proteins

E1, E2, E5
E6, E7
L1, L2
E4
E1, E2, E5
HPV vaccines

Development options

Preventive vaccines to prevent HPV acquisition – Target L1 protein with antibodies

Therapeutic vaccines for established HPV infections – Target E6 & E7 proteins with T-cells
Natural history of cell-mediated adaptive immune response to high risk HPV16

- Clearance >99%
- Minority

Immunity
- CD4+ Th1/Th2 immunity to E2, E6, E7 & L1
- CD8 immunity to E6 (E7?)
- T cells Circulate & Migrate

Immune failure
- No E6, E7 CD4+ immunity
- Impaired CD4+ T-cells
- Infrequent CD8+ T-cells
- Regulatory T-cells
Overview of different types of potential therapeutic vaccines

- Viral vector based vaccines: TA-HPV, MVA
- DNA vaccines
- DC based vaccines
- Protein vaccines: TA-CIN, E6E7 Iscomatrix
- Peptide vaccines: Minimal HLA class I binding peptides
  Synthetic Long Peptide vaccines
Long-peptide vaccine comprises both a HPV16 E7 CD8+ and a CD4+Th-epitope

Minimal CTL peptide epitope E7\textsuperscript{49-57} : **RAHYNIVTF**

Long peptide E7\textsuperscript{43-77} : GQAEPD**RAHYNIVTF**CCKCDSTLRLCVQSTHVDIR

**Th epitope**

Zwaveling et al, J. Immunol., 2002
Long peptide vaccine in HPV16 Mouse Tumour Model

GQAEPDRAHYINVTCCKCDSTLRCLVQSTHVDIR

Zwaveling et al, J. Immunol., 2002
Efficiency of processing by mouse DC of long peptide versus intact protein

**Kinetics of MHC class I antigen presentation.** To determine the efficiency of MHC class I presentation of exogenously loaded antigen, cross-presentation, DC were cultured with soluble Ovalbumin protein or the derived synthetic long peptide (OVA-31) encoding the immunodominant MHC class I epitope, SIINFEKL presented in the context of K^b_molecules. DC were pulsed for 0, 1, 2, 3, 4, 5, and 24 h with the antigens followed by extensive washing and mild paraformaldehyde fixation to inhibit further processing beyond above-mentioned timepoints. DC were then co-cultured further O/N in the presence of the CD8 T cell hybridoma (B3Z) which produces IL-2 upon recognition of K^b/SIINFEKL.
License to Kill

Clinical grade HPV16 therapeutic vaccine consists of synthetic overlapping long peptides comprising all potential CTL and Th epitopes.
<table>
<thead>
<tr>
<th>Phase</th>
<th>Patient group</th>
<th>No. of patients</th>
<th>End points</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>End stage cervical cancer</td>
<td>&gt;40</td>
<td>- Toxicity</td>
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<td></td>
<td></td>
<td></td>
<td>- immunogenicity</td>
</tr>
<tr>
<td>II</td>
<td>VIN patients</td>
<td>20</td>
<td>- immunogenicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- clinical response</td>
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</table>
Trial Design, Phase I
End-stage CxCa patients

300µg per peptide emulsified in Montanide ISA 51

### Phase I, end stage cervical cancer

<table>
<thead>
<tr>
<th></th>
<th>Before vaccination</th>
<th>After vaccination</th>
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<tbody>
<tr>
<td>medium</td>
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</tr>
<tr>
<td>E6-I</td>
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<td>E6-II</td>
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<td>E6-III</td>
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<tr>
<td>E7-I</td>
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<tr>
<td>E7-II</td>
<td></td>
<td></td>
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<tr>
<td>MRM</td>
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</tbody>
</table>

Kenter, Clin Cancer Res, 2008
Vaccination of 20 HPV16+ VIN3 patients with HPV16 SLP vaccine

HPV16-induced premalignant lesion of vulva

Non-specific symptoms: pain, itching, burning

Diagnosis: vulvoscopy, biopsies

Non-treated: can progress to cancer

Therapy: surgery, laser vaporization (mutilating)

Chronic disease: recurrence following standard treatment

Chronic disease: Only 1.3% resolves spontaneously
Vaccination against HPV-16 Oncoproteins for Vulvar Intraepithelial Neoplasia

Gemma G. Kenter, M.D., Ph.D., Marij J.P. Welters, Ph.D.,
A. Rob P.M. Valentijn, Ph.D., Margriet J.G. Lowik,
Dorien M.A. Berends-van der Meer, Annelies P.G. Vloon, Farah Essahsah,
Lorraine M. Fathers, Rienk Offringa, Ph.D., Jan Wouter Drijfhout, Ph.D.,
Amon R. Wafelman, Ph.D., Jaap Oostendorp, Ph.D., Gert Jan Fleuren, M.D., Ph.D.,
Sjoerd H. van der Burg, Ph.D., and Cornelis J.M. Melief, M.D., Ph.D.
Trial Design, Phase II, HPV16+ Vulvar Intraepithelial Neoplasia (VIN III)

Endpoints

**Immunology**
- Proliferation assay
- IFN$\gamma$ ELISPOT
- Cytokine analysis (CBA, ELISA)
- CD4/CD8 analyses (ICS)

On PBMC and Biopsies (VIN lesion, vaccination site)

**Clinical responses**
- Symptoms
- Change in lesion size
- Change in histology
- Change in HPV detection
Lymphocyte Proliferation Test (ex-vivo 6 days)

pre-vac

post-vac

cpm

cytokines (pg/ml)
HPV16-SLP vaccine induces:

- HPV16 specific T-cell proliferation in 20/20 VIN III patients.
- HPV16 specific IFNγ-producing CD4+ T-cells in 19/20 patients.
- IFNγ-producing CD8+ T-cells in 19/20 patients.
- Migration of HPV16 spec. T-cells to vaccination site in 7/18 patients.
- Complete clearance of the VIN grade III lesion in:
  - 5/20 patients, 3 months after the last vaccination,
  - 9/19 patients, 12 months after the last vaccination.
- Partial clearance of VIN grade III lesion in 5/20 patients, 12 months after the last vaccination. Overall clinical benefit in 14 of 20 patients
- Complete clearance of HPV 16 infection in 4/20 patients, 3 months after the last vaccination.
Conclusions (2)
HPV16-SLP vaccin in VIN3 patients

- HPV16 SLP vaccine is able to restore the CD4\(^+\) and CD8\(^+\) T-cell response to HPV16 E6 and E7 in VIN3 patients.
- HPV16 SLP vaccine is able to induce clinical responses in 79% of vaccinated subjects (32% PR, 47% CR).
- The strength of the HPV16 SLP vaccine-induced CD4\(^+\) T-cell response as measured by a combination of proliferation and IFN\(\gamma\) production (LST, CBA, ELISPOT) correlates with clinical responsiveness.
T-cell response after SLP® vaccination of VIN3 patients correlates with clinical outcome.
Partial clinical responses with HPV16 SLP vaccine in VIN 3 patients before 3 months FU
Complete clinical responses with HPV16 SLP vaccine in VIN 3 patients
Histology of completely cleared VIN lesion

Pre-vaccination

Post-vaccination
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Rienk Offringa

NWO
Netherlands Organisation for Scientific Research

ISA Pharmaceuticals
Immune System Activation

NEDERLANDSE
KANKERBESTRIJDING
KONINGIN WILHELMINA FONDS
Synthetic Long Peptide vaccine concept

In silicio prediction of epitopes for a particular HLA molecule

Production of overlapping long peptides

Selection by peptide binding assays

Repeat to select CD8+ and CD4+ T-cell epitopes for other HLA molecules

Processing and immunogenicity assays

Does not use full array of patient HLA molecules

Epitopes selected in vivo by patient DC

Full use of all different types of HLA molecules

Melief & Van der Burg, Nature Reviews Cancer 2008